# VALIDITY OF DETERMINATION OF DIURNAL CORTISOL PRODUCTION RATE BY ISOTOPE DILUTION METHOD

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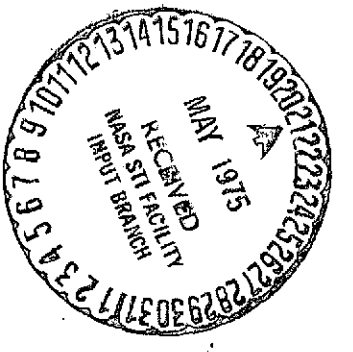
Translation of "Prawdziwość Oznaczeń Dobowej Produkcji Kortyzolu Metoda Rozcieńczeń Izotopowych," Pol. Arch. Med. Wewn. Vol. 4, No. 52, 1972 pp. 329-334.




NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON D.C. 20546 APRIL 1975

STANDARD TITLE PAGE

| 1. Report No. NASA TT F-16285 | 2. Government Accession No. | 3. Recipient's Catalog No. |
|---|---|---|
| 4. Title and Subtitle VALIDITY OF DETERMINATION OF DIURNAL CORTISOL PRODUCTION RATE BY ISOTOPE DILUTION METHOD | | 5. Report Date APRIL 1975<br>6. Performing Organization Code |
| 7. Author(s) W. Massalski, Medical Center for Postgraduate Education in Clinical Medicine, Endocrinological Clinic, Warsaw | | 8. Performing Organization Report No.<br>10. Work Unit No. |
| 9. Performing Organization Name and Address Leo Kanner Associates Redwood City, CA94063 | | 11. Contract or Grant No. NASW 2481<br>13. Type of Report and Period Covered Translation |
| 12. Sponsoring Agency Name and Address National Aeronautics and Space Administration, Washington D.C. 20546 | | 14. Sponsoring Agency Code |

15. Supplementary Notes
Translation of "Prawdziwość Oznaczeń Dobowej Produkcji Kortyzolu Metoda Rozcieńczeń Izotopowych," Pol. Arch. Med. Wewn. Vol. 4, No. 52, 1972 pp. 329-334.

16. Abstract
The author examines critically a basic assumption made in all methods attempting to determine the diurnal cortisol production rate by means of a determination of the specific activity of one of its metabolites, namely that the radioactive steroid is metabolized in the same pathways and in an identical manner as its nonradioactive counterparts and points out that the presence of the radioactive isotope may lead to considerable changes in enzymatic activity. Hence the radioactive steroid may be metabolized somewhat differently than a natural endogenous steroid. Since the specific activity of different cortisol metabolites determined in one person is not the same, the differences being as high as 60% of the value, there is no theoretical basis for an accurate determination of the diurnal cortisol production rate by the isotope dilution method. The author concludes that in spite of the reservations mentioned above the determination of the diurnal cortisol production rate by means of the isotope dilution method is useful in cases when an accurate diagnosis cannot be made using simpler methods.

| 17. Key Words (Selected by Author(s)) | 18. Distribution Statement |
|---|---|
| | Unclassified-Unlimited |

| 19. Security Classif. (of this report) | 20. Security Classif. (of this page) | 21. No. of Pages | 22. Price |
|---|---|---|---|
| Unclassified | Unclassified | 8 | |

# VALIDITY OF DETERMINATION OF DIURNAL CORTISOL PRODUCTION RATE BY ISOTOPE DILUTION METHOD

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The first tests which determined the diurnal cortisol /329* production rate in the human organism [5] were made in 1954. Two decades later a critical review of the methods used and the results obtained seems to be a good idea. With regard to biochemical studies in vitro, the general principles used in isotope dilution analysis cause no concern. They are applied on a wide scale in the determination of the losses resulting from laboratory tests. It is well known that during a chemical analysis, every steriod compound passes successively through the purification, condensation, elution and other stages. The losses in the substance studied, which frequently fluctuate and are difficult to perceive are related to these manipulations. By introducing at the beginning of the test a steroid with a labeled radioisotope which is identical to the steroid studied, it is possible to determine from the losses in the radioactive steroid the losses in the nonradioactive steroid, provided the radioactive substance accompanies the nonradioactive substance throughout the entire test. This condition is easily satisfied, because the chemical properties of the compound with the isotope are the same as those of the compound without the isotope. Both substances undergo subsequently the same changes and proportional losses. The loss in the nonradioactive (examined) substance can be calculated from the radioactive decay; the quantitative determination of the substance studied and the determination of the radioactive decay make it possible to calculate the original amount of the substance at the beginning of the test. The results obtained are reliable and they agree with the actual state. Similar reasoning was used to determine the diurnal cortisol production rate by finding the specific activity of one of its metabolites. However the starting point was the assumption that the

* Numbers in the margin indicate pagination in the foreign text.

radioactive steroid is metabolized in the same pathways and in an identical manner as its nonradioactive counterparts. This assumption is the basis of all studies striving to determine the diurnal steroid production rate by means of a determination of the specific activity of metabolites [3,4]. I did not find a single study in the entire available literature whose purpose was to validate the assumption mentioned above. In my opinion a certain amount of restraint and caution must be exercised with regard to this view. It is known that all metabolic processes in the human organism are inseparably connected with enzymatic activity. The activity of the enzymes, which depends on the hydrogen ion concentration, selected electrolytes, temperature and many other factors, may vary. The ionization of the internal medium in the cell, caused by the presence of the radioactive isotope, may lead to considerable changes in enzymatic activity. Hence a steroid particle containing a radioactive element may be metabolized somewhat differently than a natural endogenous steroid. /330

Notwithstanding the reservation mentioned above, the diurnal cortisol production rate is determined using the isotope dilution method. The oral or intravenous introduction of a radioactive hormone into the organism causes the appearance of its radioactive metabolites in the body fluids and then in the urine. The introduction of radioactive cortisol in trace amounts (1-2 μg) makes it possible to determine the so-called steroid "pool." It is assumed that the human body always contains about 1.5 mg cortisol, i.e., that this is the hormone pool in which the radioactive steroid undergoes dilution (mixing) with nonradioactive hormone particles. When the radioactivity of the steroid introduced into the organism and the ratio of the amount of radioactive to nonradioactive steroid are known, the hormone "pool" and the volume of body fluids containing the steroids studied can be determined easily.

It is assumed that a certain dynamic equilibrium occurs after some time. The curve for the radioactive steroid content in the

blood (the cortisol concentration is plotted on the ordinate and time is plotted on the abscissa) which drops rapidly for several minutes due to the diffusion into extravascular fluids acquires a tangent line which is more nearly parallel to the abscissa. The slow drop is due to the cortisol elimination and metabolism [5]. Because of the fast mixing of radioactive and nonradioactive particles, the ratios of the amounts of radioactive metabolites and the ratio of the amount of each metabolite to the amount of the radioactive precursor in the organism must be the same as the ratios of the amounts of the nonradioactive metabolites and their ratios to the amount of the precursor -- in this case the endogenous hormone. Introducing the concept of specific radioactivity, i.e. the number of pulses emitted per unit weight (for example one milligram or microgram) of the metabolite or precursor, we note that it must be the same for all metabolites and precursors found in the urine collected for a definite time period. Denoting by the starred symbol the radioactivity of the substance and by the symbol without the star the amount of the substance, we obtain the following ratios: /331

$$\frac{F^*}{THF^*} = \frac{F}{THF} \; ; \; \frac{F^*}{THE^*} = \frac{F}{THE} \quad \text{etc.}$$

and from these:

$$\frac{F^*}{F} = \frac{THF^*}{THF} = \frac{THE^*}{THE}$$

where F is the cortisol, E is the cortisone, THF is the tetrahydrocortisol and THE is the tetrahydrocortisone.

The dirunal cortisol production rate can be calculated by determining the specific activity of one metabolite when the radioactivity of the cortisol administered externally is known.

The diurnal cortisol production rate is

$$F = \frac{F^*}{\frac{THE^*}{THE}}$$

that is, the diurnal procuction rate is equal to the ratio of the radioactivity introduced to the specific activity of the metabolite. Presumably high accuracy can be attained. To this end, to determine the diurnal production rate, Pearlman introduced a special formula taking into account the differences in the molecular weight of different metabolites and also the amount of the exogenous radioactive steroid in weight units. It follows from the discussion presented that the specific activity of the metabolite is of fundamental importance in the determination of the diurnal cortisol production rate. To determine this activity, the metabolite in the urine must be isolated in a pure state and then determined in units of weight (for example using colorimetry) after which the number of pulses emitted per unit weight must be counted. The metabolite must be separated very carefully so that it is not accompanied by other steroids originating in a different metabolic cortisol pathway (which are therefore not radioactive) giving the same color reaction, and by radioactive cortisol metabolites not giving a color reaction which is specific for the metabolite studied.

Briefly, the amount of metabolite whose radioactivity will be determined in the numerator must be determined colorimetrically in the denominator. The determination of the radioactivity of at least two metabolites, usually tetrahydrocortisol and tetrahydrocortisone is recommended to check the cleanness of the separation. These metabolites are present in sufficiently large amounts even in extracts from small urine specimens. In cases when the calculated specific activities do not agree (the difference must not exceed 15%), further chromatographic purification of the sep-

arated steroid is recommended.

For many years the prevailing opinion was that the specific /332
activity of different cortisol metabolites determined in one person is the same. However it turned out that the deviations were as high as 60% of the value [1,2,3]. Thus there was no longer a theoretical basis for the accurate determination of the diurnal cortisol production rate by the isotope dilution method. It also turned out that the cortisol concentration in the blood shows not only fluctuations connected with the diurnal rhythm (highest values occur in the early morning hours and lowest values in the evening hours) but also sudden increases and decreases caused by the sporadic elimination of this steroid which has a "pulsating" character. Periods when the elimination is greater and periods in which the activity of the adrenal cortex is lower occur alternately in intervals of several tenths of minutes (Fig. 1). In earlier studies it was established that the cortisol half-life is two hours. 10-15 minutes after the pharmacological dose is introduced into the blood circulation, at most 5% of the cortisol is found in the plasma. When the radioactive cortisol is introduced intravenously in trace amounts, 30% of the administered dose can still be found in the plasma after ten minutes. The difference is probably due to the fact that the trace amounts may combine nearly completely with the protein carriers. On the other hand the pharmacological dose enters quickly enters the extravascular fluids after all protein bonds (active transport volume) were exhausted. Hence it should be emphasized that large fluctuations in the cortisol concentration have an effect on its metabolism and elimination from the body.

In the last few years it became possible to record fluctuations in steroid concentrations within the range of nano and picograms per 1 ml plasma. It is also possible to make determinations in short time intervals which are nearly continuous. As a result it was established that even small fluctuations in the

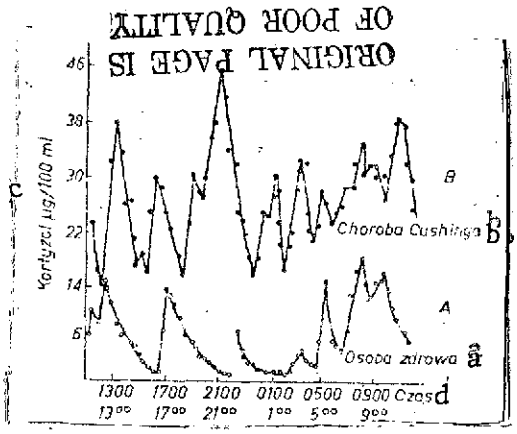


Fig. 1. Dirunal determination of cortisol in the serum from a hea healthy person and from a woman suffering from Cushing's disease. A. The periodic character of the elimination with the long quiet phases in the healthy person is noteworthy. B. A high basic elim elimination, increasing in jumps and resulting in even more in intensive elimination was noted in the case of Cushing's disease. (According to Hellman, Weitzman, Roffwarg, Afukushima, Yoshida and Gallagher*)

Key. a. Healthy person
b. Cushing's disease
c. Cortisol µg/100 ml
d. Time.

concentrations may change the metabolism. Hence if the radioactive cortisol were introduced into the body fluids throughout the entire day in a rhythm which is identical to the endogenous elimination, the calculations of the diurnal production rate would come close to the actual state. Clearly this procedure cannot be realized. In clinical studies the cortisol labeled with a radioactive isotope is administered orally or intravenously in a single dose, or in exceptional cases in the form of an uninterrupted intravenous infusion. Under these conditions the subsequent calculations include a large error (up to 60%). Modern research methods made it possible to make a series of ingenious experiments which included: /333

1. Determination of the metabolic clearance rate of cortisol (volume of body fluids expressed in liters containing an amount of cortisol eliminated from the body in a definite time period [4]).

2. Simultaneous determination of the specific activity of six cortisol metabolites in one person.

3. Simultaneous intravenous introduction of a radioactive and nonradioactive steroid in volumes simulating the sporadic

activity of the adrenal cortex in persons in whom the suprarenal gland has been removed.

The results obtained indicate that the diurnal cortisol production rate cannot be determined accurately using the isotope dilution method [5]. A dynamic equilibrium state does not exist in the so-called cortisol pool. The concentrations on which the instantaneous cortisol metabolism depends fluctuate continuously. The higher the cortisol concentration, the more THE is formed and the lower the concentration, the more THE is formed. It was not possible to obtain by any method an identical metabolism of the labelled and endogenous steroid. Moreover, on the basis of the determinations including a larger error, the statement can be made that the diurnal production rate is low, medium, high or very high.

To conclude the discussion, it is appropriate to ask and provide an answer to the question whether in view of the great inaccuracy the determination of the diurnal cortisol production rate is useful. The answer is yes in those cases in which simpler methods cannot detect the difference between the healthy and pathological state and a proper diagnosis cannot be made.

The development of a simpler method not requiring partition chromatography is a matter of considerable importance. The Endocrinological Clinic of the Medical Center for Postgraduate Education is currently engaged in research along these lines.

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